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Wing pattern and allozyme relationships in the *Coenonympha arcania* group, emphasising the *C. gardetta-darwiniana* contact area at Bellwald, Switzerland (Lepidoptera, Satyridae)

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Summary

The butterflies *Coenonympha gardetta*, *C. darwiniana* and *C. arcania* are closely related and have parapatric distributions. We studied wing pattern and allozyme variation in three sample sites near Bellwald in Canton Valais, Switzerland in an area where *C. gardetta* and *C. darwiniana* meet, and in a *C. arcania* population from northern Italy. Principal component analysis identified traits that separated the *C. arcania* population, but separate taxonomic groups could not be distinguished in the Bellwald region even when *C. arcania* was dropped from the analysis. Allozyme data showed high polymorphism characteristic of other *Coenonympha* populations, and also separated the *C. arcania* population. F-statistics revealed that the sampled populations at Bellwald, even though separated by up to 2 km, are probably subsites within a single large, demographic population. We believe *C. gardetta* and *C. darwiniana* should be considered conspecific ; *C. gardetta* is the older name. *C. arcania* should provisionally be kept distinct taxonomically, but closer study of contact regions between *C. gardetta* and *C. arcania* are required to rule out mere isolation by distance as the reason for the observed level of differentiation.

Résumé

Coenonympha gardetta, *C. darwiniana* et *C. arcania* sont des espèces proches parentes dont la répartition est parapatrique. Les auteurs ont étudié le dessin des ailes et la variation allozyme de trois lots provenant des environs de Bellwald (Valais, Suisse), région où *C. gardetta* et *C. darwiniana* sont en contact, ainsi que chez une population de *C. arcania* d'Italie septentrionale. L'analyse des principaux éléments a révélé des caractères qui séparaient la population de *C. arcania*, mais on n'a pas du distinguer de groupes taxonomiques séparés dans les biotopes de Bellwald, même lorsque *C. arcania*

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était exclu de l'analyse. Les données allozymes ont révélé un polymorphisme considérable caractéristique d'autres populations de *Coenonympha* ; elles ont également séparé la population de *C. arcania*. Les statistiques F ont prouvé que les populations-échantillons de Bellwald, même séparées par une distance de 2 km, sont probablement des sous-stations à l'intérieur d'une seule et même grande population. Les auteurs pensent que *C. gardetta* et *C. darwiniana* devraient être considérées comme co-spécifiques ; *C. gardetta* est le plus ancien nom. *C. arcania* devrait être provisoirement conservé comme espèce taxonomiquement distincte. Mais on devrait étudier de plus près les zones de contact potentiel entre *C. gardetta* et *C. arcania* afin de pouvoir exclure une simple isolation par la distance pour expliquer le degré de différenciation qu'on a constaté.

Introduction

The butterfly taxa *Coenonympha gardetta* (de Prunner, 1798), *C. darwiniana* Staudinger, 1871 and *C. arcania* (Linnaeus, 1761) are parapatric in the Alps, respectively occupying high ($\geq$ 1800 m), middle (800-2000 m), and low ($\leq$ 1000 m) elevational bands. These taxa have long been seen as closely related (e.g. Davenport, 1941). *C. gardetta* and *C. darwiniana* have contact areas where they are reported to intergrade in the southern Alps (Lepidopterologische Arbeitsgruppe der Schweiz, 1987), leading to questions about their taxonomic status as separate species. Because *C. arcania* is also parapatric, and *darwiniana* is sometimes listed in its synonomy (e.g. Forster & Wohlfahrt, 1976), it is legitimate to question the relationships among all three taxa.

In this study, we examine three populations at the contact zone between *Coenonympha gardetta* and *C. darwiniana*, and a geographically distant population of *C. arcania*. If there is indeed partial (or complete) genetic isolation between these taxa, as the current taxonomy suggests, then our contact populations should contain an excess of "pure" forms of each taxon, and few intermediates. Upon closer statistical examination, suites of diagnostic wing pattern and/or allozyme traits would appear to be correlated within individuals in these populations. However, if the major diagnostic differences between these taxa are produced by environmental conditions operating on a common genotypic array rather than by genetic differences maintained by "reproductive" barriers, then there should be no such correlations within contact populations. A similar lack of correlation would result in contact areas if hybrids between immigrants from taxonomically differentiated regional populations were not at a selective disadvantage relative to "pure" individuals. Furthermore, for traits inherited in a co-dominant Mendelian fashion

(allozymes in this study), such correlations can also be expressed in the form of *F*-statistics (Wright, 1969). These have the advantage of permitting us to enlist the analytical power of evolutionary theory to make inferences about underlying populational processes, in particular gene exchange among populations. Of course, it is gene exchange between putative taxonomic groupings that we wish to infer in the process of making taxonomic decisions. Taxonomists have traditionally done this "by eye" and therefore less reliably, especially for the traits whose genetic bases are unknown.

Methods

Mixed populations of *Coenonympha gardetta* and *C. darwiniana* were sampled in July 1991 in the vicinity of Bellwald, Switzerland, on the north slope of Canton Valais in the Rhone Valley. Population I was collected on a steep, SW-facing slope at 1700m in a meadow under an open-canopy fir forest on 13.vii.1991. Population II was sampled also at 1700 m, but 1 km N on a NW-facing, colder slope, locally above treeline, on 20.vii.1991. Population III was at 2000 m at the top of the ski lift, above the treeline in open meadow, on 21.vii.1991. The *C. arcania* population was collected at 1400 m from Monte Mottarone, near Streza, Italy, on 23.vii.1991. Individuals were haphazardly netted and stored alive under refrigeration until they could be frozen at -80C.

Wing pattern morphometrics

The taxonomic literature (Davenport, 1941 ; Higgins & Hargreaves, 1991 ; Lepidopterologische Arbeitsgruppe der Schweiz, 1987) was consulted to determine the wing pattern elements previously used to distinguish the three taxa, especially between the more similar taxa, *gardetta* and *darwiniana*. The characters proved to be mainly the size and location (relative to the wing margin) of the eyespots in the distal wing cells of the ventral hindwing, and the extent and location of the white band proximal to these spots. Spots near the apex of the ventral forewing have also been used.

Wings were removed and stored separately when specimens were prepared for electrophoresis (described below). We measured the following characters for each of eight ventral hindwing cells, along the axis of the cell : the diameter of the black center of the eyespot (absent = 0), the distance from the center of the eyespot to the wing margin (absent = unscored), the width of the white band measured to the edge

of the eyespot halo (absent = 0), and the edge of the white band to the edge of the wing (absent = unscored). We also measured the diameter of the forewing eyespots (absent = 0). We did not record from the outer wing spot rings because yellow outer rings could not be consistently distinguished when the ground colour was pale ; our scoring system thus regarded any all-yellow spots as being absent. Wing length was measured as an index of body size, and gender was recorded. The left wings were used except when one or more characters was missing due to damage, whereupon the right wings were used. Measurements were made at 20x magnification on a colour video monitor using a computerised image-analysis system. A Wild® microscope was fitted with a Sony® video camera ; this was connected to IBM PC® computer operated using the image-analysis program Optimas® (v. 3.01, BioScan, Inc.). Data collection was mechanised using a macro written in the Optimas procedural language, and measurements were saved directly to a file. We avoided characters involving colour because they were not amenable to accurate measurement using this software.

We analyzed the wing patterns using principal component analysis. This method condenses the large number of measurements per individual into a more manageable number of statistically independent characters, and is justifiable both statistically and biologically. The premise, statistically, is that some characters are likely to be correlated, whereupon they carry redundant information and should be weighted to take this into account. For example, large individuals are likely have larger measurements, and we should factor out the body size differences before we attempt to consider relative eyespot size differences. Biologically, the premise is that if the taxa are genetically isolated, then their wing patterns will have evolved independently in the separate lineages, and different pattern elements will be correlated, within lineages. The elements that are correlated would form the set of diagnostic characters useful for distinguishing the lineages. If genetic isolation were indeed involved, a small number of principal components would contain all the correlated diagnostic characters and describe most of the overall wing pattern variation among individuals, even within contact areas. But if the taxa were freely interbreeding, then characters in contact areas would tend to be assorted independently among individuals ; they would be uncorrelated. Statistically, this would be indicated if the overall wing pattern variation were spread among a larger number of principal components, evidence of independence among the wing pattern elements, and by unimodal variation along principal component axes.

Principal components analysis is, in its philosophy, what the experienced taxonomists of older generations did "by eye". The advantages of the statistical approach are three. Firstly, it is explicit, thus repeatable by others and carries known data limitations. Secondly, it is grounded in statistical theory so it takes sample size into account in a way that cannot be done properly otherwise. Finally, it can pick out much more subtle patterns than can the eye of an experienced worker, and conversely, it can demonstrate that some patterns perceived by less experienced eyes are fantasy. However, both approaches depend on the ability to pick out the "right" characters to measure, those that will give the best discrimination ; this is why, for characters in this study, we relied on the literature for the acknowledged expertise of previous workers.

We used Systat® (v.5.1, Systat Inc.) software on a Macintosh computer for the principal components analysis. We used a Pearson's *r* correlation matrix of the 28 primary measurements/individual to generate all principal components (PCs) ; gender and body size were included in these analyses as controls. The statistics require data sets without missing entries, so we were forced to omit the measures of spot or white line location for some wing cells if any individuals were absent that trait (the rejected alternative was to eliminate those individuals). We examined the loadings of characters onto each PC for suites of diagnostic characters. The proportion of the total wing pattern variance explained by each PC was used as an estimate of the overall independence of traits. We then analyzed the corresponding PC scores of each individual (i.e., the "measurement" of the individual along the PC axis, produced as a weighted combination of the original measurements in that individual) using one-way ANOVAs for to find significant variation among populations, using SuperANOVA® (v4.0 ; Abacus Concepts, Inc.) software on a Macintosh computer. The entire analysis was repeated without the *C. arcania* population to look more closely at differentiation in the Bellwald region ; here we could use a larger data set because fewer individuals were absent the eyespots or white lines in cells.

Electrophoresis

Horizontal starch gel electrophoresis was performed on head and thorax tissue using standard methods described elsewhere (Porter & Geiger, 1988 ; Porter & Matoon, 1989). We scored 19 putative genetic loci : alcohol dehydrogenase (ADH ; enzyme commission number 1.1.1.1), adenylate kinase (AK-1 ; 2.7.4.7), aldolase (ALDO ;

4.1.2.13), esterase (EST-1 ; 3.1.1.1), fumarase (FUM ; 4.2.1.2), glutamic-oxaloacetic transaminase (GOT-1, GOT-2 ; 2.6.1.1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH ; 1.2.1.12), α-glycerophosphate dehydrogenase (αGPDH ; 1.1.1.8), hexokinase (HK ; 2.7.1.1), isocitric dehydrogenase (IDH-1 ; 1.1.1.42), lactic dehydrogenase (LDH ; 1.1.1.27), malic dehydrogenase (MDH-1, MDH-2 ; 1.1.1.37) , malic enzyme (ME-1, ME-2 ; 1.1.1.40), peptidase (PEP-1 ; 3.4.1.1), phosphoglucomutase (PGM ; 2.7.5.1) and phosphoglucose isomerase (PGI ; 5.3.1.9).

We calculated standard statistics describing the extent of allozyme variability within populations. These include the mean number of alleles observed per locus, the mean heterozygosity observed per locus (H_{obs}) and that expected based on Hardy-Weinberg expectation (H_{exp}), and the percent of sampled loci that were polymorphic in the population (%P).

We describe variation among populations in two ways. First, we calculated Nei's unbiased genetic distance and produced a summary phenogram using UPGMA. This method is probably most familiar and permits comparison across a wide range of taxa. We also estimated F_{ST} (Wright, 1969) among the Bellwald populations using Weir & Cockerham's (1984) method which accounts for sampling variation. We used weighted averaging over alleles and loci, and jackknifed over loci for the error estimates. Our estimates were interpreted using the relationship $M \approx (1/F_{ST}-1)/4$, where M is a gene flow parameter describing the effective number of individuals moving among populations each generation (Cockerham & Weir, 1993). A fundamental result in theoretical population genetics is that when $M > 0.5$, then gene flow produces substantial genetic similarity among populations at neutral loci (Wright, 1931). We will not provide the theoretical and statistical details here ; interested readers may consult population genetic texts (e.g. Hartl & Clark, 1989) for introductory concepts, Cockerham & Weir (1993) and references therein for current statistical theory, and Porter (1990) and Porter & Geiger (1988 ; 1995) for examples of applications to butterfly populations.

Results

Wing pattern morphometrics

Coefficients of variation (c.v.) for each trait are shown for each population and for all populations combined (Table 1). A c.v. of 0.1 means that the standard deviation is 10% of the mean for the trait, a reasonably

Table 1

Coefficients of variation for the ventral wing pattern characters used in this study. Missing values indicate the trait was not present in the population

character	Bellwald I	Bellwald II	Bellwald III	Mt. Mottarone	total
n	26	9	35	17	87
forewing length	0.16	0.11	0.14	0.14	0.15
forewing spot diameter					
1	5.00			4.24	8.67
2	1.36	0.80	0.98	0.68	1.00
hindwing spot diameter					
1	0.22	0.19	0.32	0.30	0.33
2	0.29	0.56	0.33	0.70	0.44
3	0.39	0.18	0.22	0.28	0.31
4	0.23	0.13	0.21	0.21	0.29
5	0.21	0.13	0.16	0.37	0.29
6	0.39	0.14	0.40	1.00	0.51
white line width					
1	0.54	0.57	0.51	0.44	0.60
2	0.42	0.50	0.32	0.33	0.41
3	0.39	0.52	0.32	0.26	0.39
4	0.47	0.79	0.55	0.23	0.55
5	0.53	1.01	0.77	0.44	0.63
6	0.49	0.48	0.61	0.59	0.57
7	1.15	0.65	1.31	0.71	1.04
8	3.49		3.02	2.48	3.16
spot location					
1	0.43	0.45	0.54	0.44	0.51
2	0.17	0.09	0.22	0.58	0.30
3	0.16	0.10	0.22	0.18	0.19
4	0.15	0.12	0.17	0.40	0.27
5	0.17	0.08	0.22	0.41	0.28
6	0.37	0.11	0.32	1.07	0.50
white line location					
1	0.41	0.31	0.31	0.53	0.42
2	0.17	0.26	0.16	0.37	0.23
3	0.19	0.29	0.19	0.33	0.27
4	0.18	0.37	0.26	0.41	0.39
5	0.20	0.37	0.26	0.50	0.41
6	0.37	0.21	0.32	0.62	0.48
7	1.03	0.20	0.93	0.65	0.86
8	2.44		2.59	1.53	2.26

high level of variability. Variability is high in the wing pattern traits taxonomists have identified as important, being generally higher than c.v. $= 0.2$, and is comparable among populations. Some traits, particularly those present in only a few individuals, showed c.v. > 1, an extreme level of variability. This included pattern elements in hindwing cells 7 and 8, and in the forewing spots.

First consider variation in all populations. Eigenvalues and the proportion of total variance explained by the first ten PCs are in Table 2. The character loadings of the first six PCs are given in Table 3. PC 1 loaded highly for most characters, especially for forewing length, and we interpret it as a general body size character. Note that several traits did not load here, indicating that these varied relatively independently of body size (this points out the hazards of an alternative approach : dividing all measurements by body size for standardisation before statistical analysis). These include the spot diameter in cell 6 and the white line width in cell 1. PC 2 describes an inverse ratio of spot diameter and white line width, and is largely independent of body size. PC 3 mainly describes the shape of the white line as it traverses the cells, with loadings being positive in the first cells and negative in the later cells ; the spot diameters in cells 2 and 6 and the width in cell 2 also load here. This PC captures a previously reported taxonomic difference between *arcania*, with a line that narrows posteriorly, and the others, with a line of relatively constant width. PCs 4 and 5 describe subtle relationships between spot diameters, white line widths and their locations in several cells. PC 6 describes sexual dimorphism, and characters that also load here include the spot diameter of cell 6 and the white line width in cell 8.

Table 2

Principal component analysis with all population included.
Eigenvalues of the first ten PCs
and the percent of the total variance explained

PC	Eigenvalue	% variance explained
1	5.860	29.3
2	3.101	15.5
3	2.203	11.0
4	1.442	7.2
5	1.437	7.2
6	1.313	6.6
7	1.086	5.4
8	0.578	2.9
9	0.567	2.8
10	0.407	2.0

Figure 1 shows differentiation among populations in PC scores. We found significant interpopulational differences in PC 1 (ANOVA ; $F_{3,72} = 14.958$; $P < 0.0001$), with two groups segregating in the followup test (Duncan's New Multiple Range Test, $P < 0.05$) : the

Table 3

Character loadings on the first six principle components using all populations.
Numbers for traits refer to wing cells.
Loadings are the extent to which a character is correlated with the PC

trait	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
gender	0.020	-0.249	0.076	-0.079	0.262	-0.843
forewing length	0.824	0.092	0.283	-0.133	-0.150	-0.018
forewing spot diameter						
2	0.313	0.317	-0.236	0.021	0.230	-0.122
hindwing spot diameter						
1	0.729	0.408	-0.160	-0.115	0.084	0.204
2	0.256	0.304	0.389	0.521	0.366	-0.141
3	0.516	0.486	0.086	0.070	0.519	0.091
4	0.782	0.411	-0.223	-0.043	0.145	0.018
5	0.684	0.321	-0.203	0.243	0.061	0.053
6	0.040	0.053	0.402	0.534	-0.407	0.356
white line width						
1	0.072	-0.305	0.772	-0.172	0.374	-0.010
2	0.340	-0.596	0.501	-0.119	0.052	0.146
3	0.617	-0.561	-0.111	-0.339	-0.060	0.077
4	0.495	-0.435	-0.535	-0.232	0.119	0.154
5	0.411	-0.710	-0.055	0.090	0.163	0.213
6	0.479	-0.547	0.043	0.331	0.254	0.143
7	0.565	-0.394	-0.253	0.330	0.055	-0.084
8	0.200	-0.392	-0.210	0.480	-0.313	-0.430
indwing spot location						
3	0.797	0.012	0.077	0.090	-0.401	-0.118
white line location						
2	0.555	0.244	0.600	-0.255	-0.277	-0.130
3	0.832	0.189	0.010	-0.199	-0.321	-0.221

Mottarone and Bellwald II population both had larger mean body sizes than the other populations (Figure 1a). The Mottarone population (*arcania*) differed from the others in PC 3 (ANOVA ; $F_{3,72} = 17.164$; $P < 0.0001$), indicating a difference in white line shape between these groups (Figure 1b). PC 6 also showed significance (ANOVA ; $F_{3,72} = 3.233$; $P = 0.027$), but we attribute this to a difference in sex ratios among populations. These differences demonstrate that the *arcania* population is phenotypically different from the remaining populations, and by themselves, suggest a possibility of genetic isolation. Means of PCs 2, 4 & 5 were not significantly different among populations, and we consider them to represent patterns of variation common to all populations.

The differences between *arcania* vs. Bellwald populations could potentially obscure more subtle differentiation between *gardetta* and *darwiniana* in the contact zone, so it is appropriate to reanalyze the data dropping the Mottarone population.

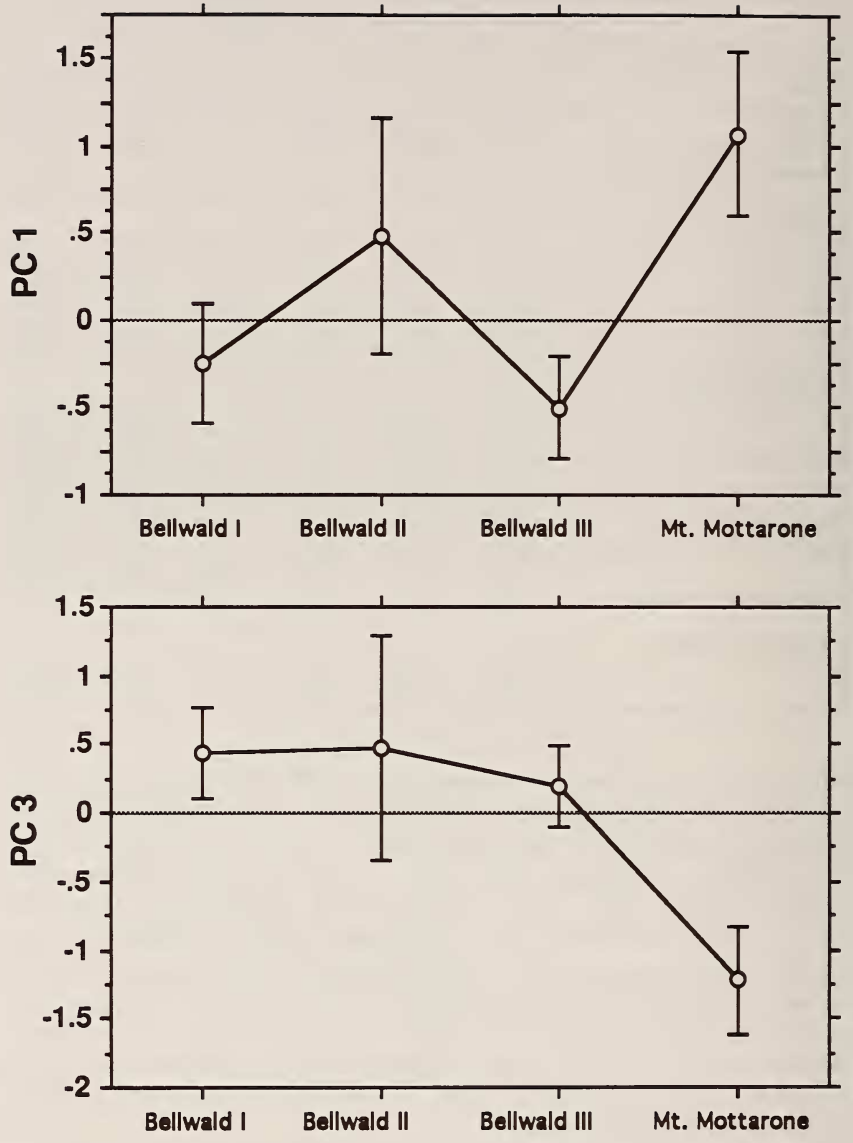

Fig. 1. Principal component means (95% c.i.) showing differences among populations. (a) PC 1, describing body size ; (b) PC 3, describing the shape of the white line.

Table 4

Principal component analysis with only Bellwald populations.
Eigenvalues of the first ten PCs
and the percent of the total variance explained

PC	Eigenvalue	% variance explained
1	8.718	36.3
2	3.946	16.4
3	2.322	9.7
4	1.701	7.1
5	1.507	6.3
6	1.038	4.3
7	0.740	3.1
8	0.648	2.7
9	0.616	2.6
10	0.514	2.1

Here we concentrate on the *gardetta/darwiniana* sampling sites at Bellwald, excluding *arcania*. Eigenvalues and the proportion of total variance explained by the first ten PCs are in Table 4. The character loadings of the first six PCs are given in Table 5 ; it is important to remember that these PCs describe different combinations of characters than those in the previous analysis. PC 1 again loaded highly for most characters, especially for forewing length, and we interpret it again as a general body size character. The forewing spot diameter and the several of the white line widths vary little with body size, nor is there sexual size dimorphism. PC 2 shows strong negative loadings for white line width and weak positive loadings for spot diameters. We interpret it as a white line width parameter that is in relative agreement with a diagnostic character often used to separate *gardetta* and *darwiniana*. PC 3 describes a differentiation pattern in inter-cell ratios of spot and white line sizes — at one extreme are *darwiniana*-like individuals with the middle cells having relatively smaller spots and extreme cells having wider lines, at the other are individuals with the reverse. PC 4 describes sexual dimorphism, with hindwing spot 6 being larger in males and the white band slightly more centrally located in females. PCs 5 & 6 describe mainly relationships among spot sizes.

We found significant differences among populations only in PC 4 (ANOVA ; $F_{2,57} = 5.093$; $P = 0.009$), and this was attributed to the different sex ratios of these population samples. The difference in body size from the previous analysis was not quite significant here (ANOVA ; $F_{2,57} = 2.736$; $P = 0.073$). Distributions for all three Bellwald populations along PC axes 2 & 3 are shown in Figure 2 ; these axes describe

Table 5

Loadings for the first six PCs for the *gardetta-darwiniana* contact area only

trait	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
gender	-0.009	-0.256	0.053	-0.758	0.336	-0.173
forewing length	0.870	0.003	0.084	-0.007	-0.219	0.063
forewing spot diameter						
2	0.169	0.232	0.208	0.002	0.657	-0.569
hindwing spot diameter						
1	0.630	0.271	0.260	0.229	-0.033	0.177
2	0.445	0.276	0.308	-0.299	0.368	0.358
3	0.451	0.267	0.653	0.035	0.202	0.200
4	0.801	0.233	0.310	0.147	0.215	0.035
5	0.771	0.156	0.005	0.283	0.294	0.090
6	0.447	0.092	-0.441	0.583	0.268	-0.084
white line width						
1	0.383	-0.288	0.610	-0.260	-0.212	-0.219
2	0.541	-0.643	0.230	-0.011	-0.129	-0.156
3	0.298	-0.812	0.079	-0.030	-0.205	-0.077
4	-0.087	-0.794	0.111	0.291	0.128	-0.240
5	0.189	-0.795	0.120	0.136	-0.018	0.156
6	0.312	-0.641	0.184	0.093	0.166	0.365
7	0.318	-0.591	-0.074	0.039	0.367	0.033
8	0.048	-0.468	-0.597	-0.310	0.313	0.332
hindwing spot location						
2	0.869	0.055	-0.220	0.042	-0.105	-0.196
3	0.847	-0.094	-0.372	0.034	0.038	-0.112
4	0.863	0.053	0.126	0.099	-0.218	0.004
5	0.850	-0.067	-0.287	0.021	-0.139	-0.078
white line location						
2	0.808	0.257	0.042	-0.261	-0.258	-0.057
3	0.853	0.135	-0.253	-0.328	-0.074	-0.017
5	0.780	0.135	-0.430	-0.274	0.012	0.012

most of the *gardetta-darwiniana* diagnostic characters. Despite the numerous individuals that appear to have *gardetta* and *darwiniana* characteristics, there is unimodal variation and no evidence from wing pattern that these taxa are genetically isolated.

Allozyme differentiation

Allelic frequencies for variable loci are given in Table 6. The loci EST-1, GAPDH and αGPDH were monomorphic. Summary statistics describing genetic variability within populations is given in Table 7. Variability is remarkably high, a common phenomenon in butterfly populations, including *Coenonympha* (Porter & Geiger, 1988 ; Porter & Matoon, 1989), and indicates that these populations are quite large and have not been through population bottlenecks in their recent past.

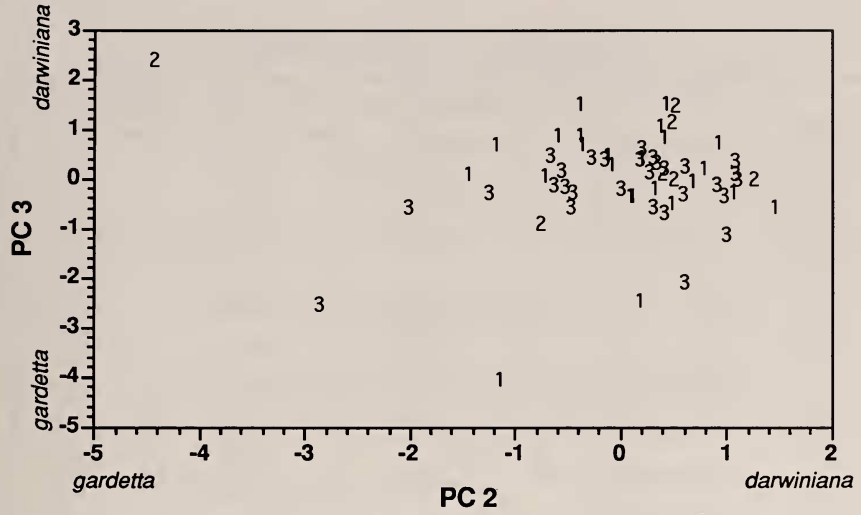

Fig. 2. Distributions of individuals from the Bellwald populations along PC axes 2 & 3 are unimodal. 1 — from Bellwald I ; 2 — Bellwald II ; 3 — Bellwald III. PCs are standardised to have their means at zero and scale in units of standard deviations. The populations do not differ significantly along either axis.

Differentiation among populations is described graphically using a distance Wagner procedure (Farris, 1972) on a matrix of pairwise Rogers' (1972) genetic distances (Figure 3a), and using UPGMA cluster analysis on a matrix of Nei's (1978) unbiased genetic identities (Figure 3b). The Mottarone *arcania* population segregates from the others, at a level suggesting that it may be genetically isolated. The remainder cluster closely, at levels commonly observed among conspecific populations in other butterfly taxa.

The high similarity within the Bellwald group permits closer populational analyses using F-statistics. Within the Bellwald population group, we obtained an estimate of θ $(= \hat{F}_{ST}) = -0.00223 \pm 0.00127$ (s.d.), averaged over alleles and loci. This slightly negative value is a result of sampling error and shows that θ is not significantly different from zero, indicating that there is no appreciable genetic differentiation among sites. This is perhaps more easily understood when translated to a gene flow estimate ($\hat{M}$) using $\hat{M} = (1/\theta - 1)/4$, yielding a value of $942 \leq \hat{M} \leq$ panmictic (95% c.i.). This large number of individuals exchanged among sites each generation suggests that the sampled "populations" are effectively sub-sites within a single, larger panmictic population in the Bellwald area.

Table 6

Allele frequencies for variable loci, by population.
Sample sizes for each locus in brackets

	Monte Mottarone	Bellwald I	Bellwald II	Bellwald III
FUM	[17]	[23]	[9]	[31]
A	0.029	0.065	0.111	0.016
B	0.676	0.674	0.778	0.774
C	0.294	0.261	0.111	0.210
GOT-1	[17]	[26]	[9]	[34]
B	0.118	0.135	0.278	0.074
C				0.044
D	0.118	0.731	0.611	0.691
F	0.471	0.038		0.029
G		0.058	0.056	0.118
H	0.294	0.038		
I			0.056	0.044
GOT-2	[10]	[14]	[8]	[16]
A		0.071	0.312	0.156
B	1.000	0.929	0.688	0.844
HK	[18]	[26]	[9]	[35]
A	0.028			
B	0.972	1.000	1.000	1.000
IDH-1	[16]	[26]	[9]	[34]
A	0.969	1.000	1.000	1.000
C	0.031			
LDH	[6]	[21]	[7]	[25]
A	1.000			
B		0.238	0.500	0.360
D		0.762	0.500	0.640
MDH-1	[17]	[26]	[9]	[35]
A	0.029	0.404	0.500	0.371
B	0.971	0.596	0.500	0.629
MDH-2	[18]	[26]	[9]	[35]
A	1.000	1.000	1.000	0.986
B				0.014
ME-1	[8]	[16]	[9]	[23]
A	1.000	0.969	1.000	1.000
B		0.031		
ME-2	[8]	[16]	[9]	[24]
A	0.062			
B	0.938	1.000	1.000	1.000
PEP-1	[17]	[26]	[9]	[34]
A	0.088	0.058	0.056	0.029
B	0.618	0.635	0.778	0.676
C	0.294	0.308	0.167	0.294
PGI	[18]	[26]	[9]	[35]
B	0.083	0.077	0.111	0.014
C				0.014
D	0.583	0.173	0.167	0.129
E	0.028			
F	0.278	0.615	0.611	0.643
G	0.028	0.019		
H		0.096	0.111	0.143
I		0.019		0.029
J				0.029
PGM	[18]	[26]	[9]	[35]
A	0.111	0.154		0.143
B	0.111			
D	0.417	0.827	0.944	0.800
E	0.361	0.019		0.057
G			0.056	

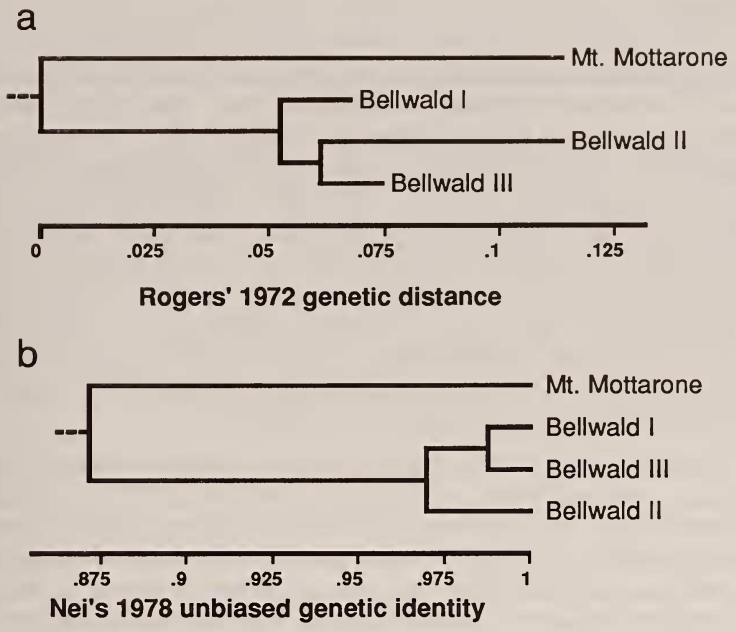

Fig. 3. Genetic differentiation among populations, described by (a) a distance Wagner procedure (FARRIS, 1972) using ROGERS' (1972) genetic distance, rooted using the mid-point of the longest path ; (b) UPGMA using Nei's unbiased genetic identity.

Table 7

Genetic variability within populations (s.e.).
A : mean alleles per locus ; %P : percent of loci that were polymorphic ;
H_{obs} : the observed proportion of heterozygotes, averaged over loci ;
H_{exp} : the proportion of heterozygotes expected from Hardy-Weinberg genotypic proportions, averaged over loci.
Standard errors calculated using a jackknife procedure, over loci

	population	A	%P	H_{obs}	H_{exp}
1	Monte Mottarone	2.27 (0.02)	60.0 (0.9)	0.229 (0.005)	0.199 (0.004)
2	Bellwald I	2.33 (0.03)	60.0 (0.9)	0.187 (0.004)	0.207 (0.004)
3	Bellwald II	2.00 (0.02)	53.3 (0.9)	0.213 (0.004)	0.212 (0.004)
4	Bellwald III	2.47 (0.03)	60.0 (0.9)	0.192 (0.004)	0.213 (0.004)

Discussion

Both morphological and allozyme analyses show that the Monte Mottarone *C. arcania* population is different from the others, but that continuous variation exists between *C. gardetta* and *C. darwiniana* at Bellwald. The morphometric analyses isolated essentially the same suites of "diagnostic" character states that taxonomists have used, showing that the results are not spurious as has been suggested in similar studies (Hammond, 1985). Neither is the high genetic similarity a spurious result of low variability : genetic variability is high enough that if two species have been coexisting at Bellwald, then genetic drift should have already differentiated the populations (see Porter & Geiger, 1995 for a discussion of this effect). There is no evidence to suggest that these are distinct species at Bellwald.

The *C. gardetta-darwiniana* contact region is similar to contact regions between *C. tullia* (Müller, 1764) taxa in western North America. *C. tullia*-group taxa in California, Nevada and Oregon differ in eyespot size and number, dorsal and ventral ground colour and the shape and placement of the white lines, and were distributed among several nominal species. However, populations are highly variable, and taxonomists, with the notable exception of Davenport (1941), had tended concentrate on widely separated localities, ignoring contact areas and intrapopulational variability. When contact areas were examined, and allozyme data used as corroborating evidence, it was found that wing patterns intergraded, gene flow was high, and no genetic isolation was apparent (Porter & Geiger, 1988). Instead, the geographic variation in wing pattern was attributed to unknown selective and/or historical factors, being maintained in the face of strong gene flow between the taxonomically recognised forms. In one remarkable example, a population on coastal dunes was sharply differentiated in wing pattern from one only 8 km away on a hilltop, yet the allozyme data could only be reasonably interpreted as showing evidence of strong gene flow between them (Porter & Matoon, 1989).

The allozyme data indicate that the *C. gardetta-darwiniana* population at Bellwald is probably quite large and demographically continuous over an area of several km^2. This area encompasses the respectively "typical" subalpine and alpine habitats of *C. darwiniana* and *C. gardetta*, and is consistent with the interpretation that no genetic isolation exists between them. The implication is that individuals are quite mobile, readily moving distances of 2 km or more in their lifetimes. Whereas this should obviously be verified with a demographic study, we point out that marked *C. tullia californa* Westwood, 1851 in Cali-

fornia have been recaptured at distances >1 km (Weissman, 1972), and stray individuals have been seen in the Central Valley (Davis area) at least 40 km from potential source populations (Shapiro, 1982; Porter, pers. obs.).

We believe *C. gardetta* and *C. darwiniana* should be considered conspecific and the younger name, *darwiniana*, be placed in synonymy. This decision is based on the high genetic and phenotypic variability in the contact zone without apparent isolation, and remains subjective pending verification by closer demographic studies there. However, the patterns are strongly suggestive, and the onus now properly belongs on a splitter to demonstrate that *C. gardetta* and *C. darwiniana* are genetically isolated, rather than being extremes of a cline as we believe they are. Indeed, the high gene flow estimate would argue that *darwiniana* not even be used as a subspecies name, because individuals from populations of "*darwiniana*" wing phenotype are likely to be much more closely related in pedigree to those from "*gardetta*" populations directly upslope than to butterflies from populations with "*darwiniana*" phenotypes at more distant localities. A minor difference between *C. gardetta* and *darwiniana* in the male genitalia has been identified (Higgins, 1975), but Davenport (1941) found the genitalia to be quite variable. Regardless of the validity of this putative difference, small differences in the genitalia *per se* have little value as evidence of reproductive isolation between parapatric taxa (Porter & Shapiro, 1990).

The possibility also remains that *C. arcania* and *C. gardetta* (+ *darwiniana*) are conspecific, as we have only studied widely separated populations and these taxa too may intergrade in their contact areas. However, both the allozyme and wing pattern differentiation in our limited data are consistent with differences between closely related sympatric taxa, and we believe their continued designation as species — the *status quo* — is appropriate pending publication of studies done in contact areas. Anticipated results from a current study of the *C. arcania* group, on a larger geographic scale than ours (Wiemers, 1994), will bring us much closer to the solution of this problem.

Systematists of *Coenonympha* have long relied heavily on qualitatively described wing pattern dimensions as characters, even while lamenting their intra- and interpopulational variability (Davenport, 1941). Species-level taxonomic decisions in *Coenonympha* could be greatly illuminated if we understood more about how wing pattern is developmentally, genetically and ecologically controlled, because it would help us determine the extent to which character variation could be used

as markers for more profound genetic differentiation. Studies of the wing patterns of *Coenonympha* indicate that several taxa have clines in spot pattern, with spot size diminishing with increasing elevation (Brunton *et al.*, 1991) or latitude (K. Porter, 1980 ; Dennis *et al.*, 1986) ; these patterns also appear to occur in North American *C. tullia* but have not yet been studied quantitatively. Though the eyespots of the Nymphalidae are probably serially homologous (Nijhout, 1991), multivariate statistical analyses have demonstrated that the pattern elements in different wing cells are largely developmentally independent (Nijhout, 1985 ; Dennis *et al.*, 1984). Dennis *et al.*, (1986) proposed that geographic variation in spot size in *C. tullia* is attributable to parallel variation in selection pressures imposed by different suites of predators or predator abilities relative to gross habitat type. The alternative that one might infer from clinal variation, that spot pattern is not genetically controlled at all, seems unlikely given the weaknesses of correlations of spot sizes within individuals and the heritability of spot pattern in other Satyrinae (Brakefield & Noordwijk, 1985). It would also be difficult to credibly explain, without invoking adaptive genetic differences, why spot size gets smaller with increasing elevation in *C. gardetta*, but larger with increasing elevation in *C. corinna* and North American *C. tullia*, even though the ecological causes of these putative adaptations remain elusive. This is not to exclude an important role for environmental effects : polyphenism is apparent in several satyrine taxa, e.g. *Bicyclus* (Brakefield & Reitsma, 1991) and *C. tullia* (Weissman, 1972), indicating the presence of genotype by environment interactions in the control of wing pattern. Though *C. arcania* and *C. gardetta* usually have only a single annual generation, similar underlying genetic mechanisms could well be influencing their wing patterns. Unfortunately, there are substantial technical difficulties to surmount before these issues can be quantitatively studied in *Coenonympha* and, especially, before they can be used to truly test assumptions underlying species-level taxonomic decisions.

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